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## Ecological dynamics of two remote Alpine lakes during ice-free season

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### ABSTRACT

We studied hydrochemistry and plankton dynamics in two remote Alpine lakes: lake Nivolet superiore (2530 m asl) and lake Trebecchi superiore (2729 m asl) in the Gran Paradiso National Park (Western Italian Alps) in summer 2009. The aim of this study was to enhance the understanding of natural ecological dynamics in the pelagic habitat of Alpine lakes by enlarging the number of biotic and abiotic variables usually considered to this end and by increasing the frequency of samplings, generally low in remote lakes. During the eight samplings performed in 2009, chemical and physical variables were measured both in situ and in the laboratory. We also followed the dynamics of all the compartments of the naturally simplified trophic chain of the two lakes from pico-prokaryotes to phytoplankton and zooplankton. Our results confirm the oligotrophic, close-to-pristine state of lake Nivolet and lake Trebecchi as they are not affected by hydromorphological alterations, they are naturally fishless and are not sensitive to acidification risk and acidity pulses. On the other hand, the two lakes have distinct abiotic conditions due to their glacial origin and to the lithological composition of their watersheds. We found some differences in the spatial variation of pico-prokaryotes, phytoplankton and zooplankton due to the different mixing regimes and maximum depth of the two lakes. Conversely, temporal patterns were similar in both lakes, related to ecological interactions and to changes in the abiotic conditions. The rapid succession of events in extreme ecosystems, such as the Alpine lakes studied here, confirm the predominant role of external environmental factors (e.g., the duration of the ice-free season) and of ecological interactions among different trophic compartments. This research underlines the importance of seasonal niche partitioning among organisms with different size.

Key words: lake chemistry, bacteria, phytoplankton, zooplankton, Gran Paradiso National Park.

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### INTRODUCTION

Alpine lakes are remote ecosystems, exposed to harsh climatic conditions (Straskabova *et al.*, 1999a; Catalan *et al.*, 2006). Under the ice, a peculiar overwintering community can develop (Salonen *et al.*, 2009), but a large variety of aquatic organisms are constrained to complete their life cycle within a short ice-free season before the lakes freeze and turn into dark heterotrophic systems, isolated from the surrounding terrestrial habitats for most of the year.

The rapid environmental changes of Alpine lakes lead to non-stationary summer dynamics (Ventura *et al.*, 2000), so that frequent samplings are necessary to delineate a realistic pattern of plankton communities. Although the main seasonal patterns have been described for high altitude lakes in the Alps (Pechlaner *et al.*, 1970; Tilzer and Schwarz, 1976; Mosello *et al.*, 1992; Callieri and Bertoni, 1999; Winder *et al.*, 2003; Salcher *et al.*, 2010) and in other mountain regions (Capblancq and Laville, 1983; Mcknight *et al.*, 1990; Pienitz *et al.*, 1997a, 1997b; Ventura *et al.*,

2000; Ellis *et al.*, 2003), the understanding of their seasonal variability is still partially limited by the piecemeal approach which is commonly used and by the specific ecological features of the lakes, which are often directly or indirectly impacted by human activities (e.g., acidified lakes or lakes stocked with alien fish) (Mosello *et al.*, 1992; Winder *et al.*, 2003). Indeed, high-altitude lakes can be frequently exposed to several anthropogenic threats, such as water exploitation (CIPRA, 1992; Godlewska *et al.*, 2003), alien species introduction (Eby *et al.*, 2006; Magnea *et al.*, 2013), climate change (Rogora *et al.*, 2003) and long range atmospheric transport of air pollutants (Ørbæk *et al.*, 2007). As a result, it is difficult to find a truly pristine lake in some mountain regions. For example, in the mountain lakes of Western United States, Bahls (1992) estimated that 95% of lakes deeper than 3 m contained non-native trout. Furthermore, only one or few ecological compartments are studied at a time (Mcknight *et al.*, 1990; Callieri and Bertoni, 1999), so that in general it is difficult to fully understand the lake ecosystem functioning.

To overcome at least some of these shortcomings, we planned an intensive sampling campaign (fortnightly from July to October) in two high mountain lakes located in the Gran Paradiso National Park, providing the measurement of climatic, chemical and physical variables and the study of the pico-prokaryotic, phytoplanktonic and zooplanktonic communities. The aim of this study was to enhance the understanding of natural ecological dynamics in the pelagic habitat of Alpine lakes by enlarging the number of biotic and abiotic variables usually considered to this end. We chose lake Nivolet *superiore* (NIV) and lake Trebecchi *superiore* (TRS) as study sites because they are naturally fishless, and, as shown by a first study which included many lakes of the Gran Paradiso National Park (Tiberti *et al.*, 2010), they resulted not sensitive to acidification risk and to acidity pulses and not significantly affected by long range transport of atmospheric pollutants, such as nitrogen compounds. We expected to find differ-

ent ecological assemblies or dynamics in these two lakes since they offer distinct abiotic conditions due to their distinct glacial origin and to the different lithological composition of their watersheds.

## METHODS

### Study area

Lake Nivolet *superiore* (45° 28' 54" N, 07° 08' 41" E) and TRS (45° 30' 07" N, 07° 08' 40" E) belong to the catchments of the Dora di Savarenche stream, entirely located in the Gran Paradiso National Park (Western Italian Alps) (Fig. 1). The lakes are not affected by hydromorphological alterations, are larger than one hectare and are both located above the local timberline, with their watershed belonging to the Alpine and nival belts.

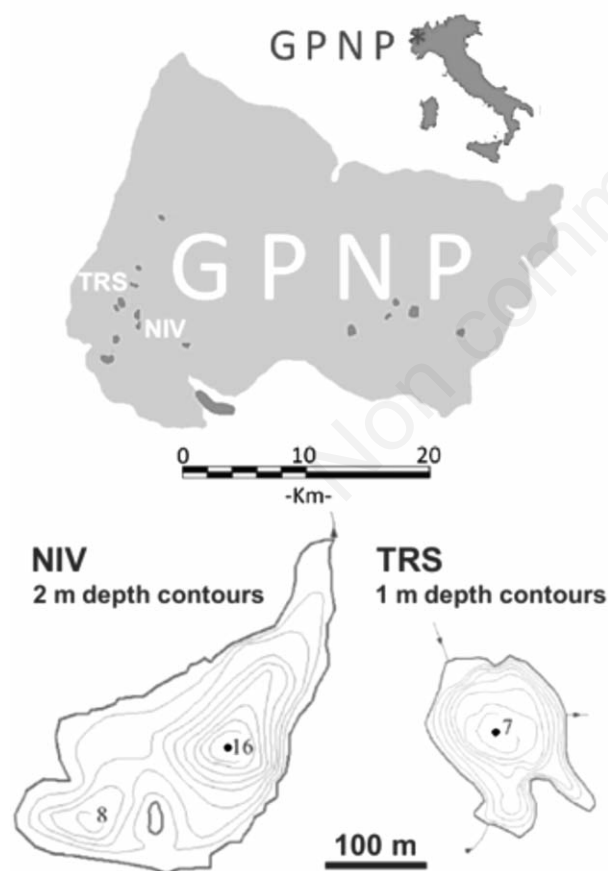
Lake Nivolet *superiore* is a tarn with its water level risen by a rock slide dam, filling the higher glacial depression of Savarenche valley (Tiberti *et al.*, 2010). The lake free surface altitude is 2530 m, the maximum depth is 17.1 m, the area is 3.45 ha, the perimeter is 986 m and the volume is  $1.611 \times 10^{-4}$  km<sup>3</sup>. The catchment area is 29.1 ha and is entirely dominated by acidic gneiss (Compagnoni *et al.*, 1974); the basin is covered by debris and bare rocks (74.8%) and by Alpine meadows (12.9%).

Lake Trebecchi *superiore* fills a shallow depression produced by glacier erosion (Tiberti *et al.*, 2010). The lake free surface altitude is 2729 m, the maximum depth is 7.5 m, the area is 1.42 ha, the perimeter is 565 m and the volume is  $0.489 \times 10^{-4}$  km<sup>3</sup>. The catchment area is 23.7 ha and is dominated by a thick covering of calcareous schists (Compagnoni *et al.*, 1974); the basin is covered by Alpine meadows (77.6%) and rocks (18.0%).

### Sampling and analytical methods

#### Sampling strategy and technique

During the ice-free period eight samplings were performed fortnightly in NIV and TRS from July to October 2009. Each measurement was carried out from 11 a.m. to 4 p.m., close to the deepest point of the lakes, marked by a moored floating buoy. We designed a standard sampling procedure which consisted of three separate actions: i) measurement of the temperature and oxygen profiles; ii) zooplankton sampling (depth-integrated sampling); and iii) water sampling for chemical analyses (depth-integrated sampling), pico-prokaryotes (sampling at three depths) and phytoplankton (depth-integrated sampling). The collected water samples were analysed at the laboratories of the *Istituto per lo studio degli ecosistemi, Consiglio Nazionale delle Ricerche* (CNR ISE), Verbania Pallanza, and at the *Dipartimento di Scienze della Terra e dell'Ambiente (DSTA)* of the University of Pavia.



**Fig. 1.** Map of the Gran Paradiso National Park (Western Italian Alps) and bathymetries of lakes Nivolet *superiore* (NIV) and Trebecchi *superiore* (TRS). Black dots indicate the sampling sites at maximum depth point for each lake.

### Physical and chemical parameters

*In situ* temperature and oxygen profiles were obtained using a multiparametric probe, Idroprobe IP145D (IDROMAR Srl., Genova, Italy). Water samples for chemical analysis (500 mL) were collected with a horizontal Van Dorn bottle at 3 m intervals in NIV and at 2 m intervals in TRS at the point of maximum depth and were integrated from surface to the bottom. Water samples were stored in the dark at 4°C and analysed at the laboratories of CNR-ISE in Verbania Pallanza, following Tartari and Mosello (1997). Water samples were analysed for pH, conductivity, alkalinity (acidimetric titration, Gran's method), total phosphorus, reactive phosphorus and reactive silica (spectrophotometry: spectrophotometer SAFAS UV mc2), major anions ( $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ), major cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{N-NH}_4^+$ ) [ion chromatography after filtration on 0.8  $\mu\text{m}$  pore size filters: ion analysers Dionex series DX320 for anions and Dionex series ICS3000 for cations (Thermo Fisher Scientific Inc., Sunnyvale, CA)], total nitrogen (TN), and total organic carbon (TOC) [catalytic combustion with a Skalar Formacs series TOC/TN Analyser (Skalar, Breda, The Netherlands)]. Organic nitrogen (ON) concentrations were calculated from the difference between TN and inorganic nitrogen ( $\text{IN}=\text{N-NO}_3^-+\text{N-NH}_4^+$ ) concentrations.

In order to better characterise the physical and chemical properties of the lakes, we used meteorological data from the weather station of lake Serrù (45° 27' 33.13" N, 7° 7' 32.86" E, 2275 m asl) and data on precipitation chemistry from the sampling site of La Thuile (45° 43' 26" N, 6° 55' 55" E, 1740 m asl). The meteorological station of lake Serrù is located within the Gran Paradiso National Park, near the lakes under study (at 2.3 km from NIV and 4.5 km from TRS). The atmospheric deposition sampling site of La Thuile is about 30 km NW of the lakes; 23 precipitation samples collected weekly throughout 2009 were analysed at the CNR ISE chemical laboratory following the same methods used for the lake water.

A comparison between the sum of anions and cations and between measured and calculated conductivity was performed for each analysis. Further quality-assurance measures involved the use of control charts, the analysis of synthetic samples on a regular basis and participation in inter-laboratory comparisons on rain and surface water analysis (Marchetto *et al.*, 2010).

### Pico-prokaryotes

Samples for bacteria and picocyanobacteria analysis were collected with a horizontal Van Dorn bottle at different depths: on the surface, at mid depth, and close to the bottom. To describe the dynamics of pico-prokaryotes, we frequently measured their abundance and biovolumes. The samples were kept at low temperature in 200 mL dark glass sterilised bottles and immediately fixed adding filtered (0.2

$\mu\text{m}$ ) formalin solution (2% final concentration). Two subsamples (0.5–1.0 mL) were stained with 4',6-diamidino-2-phenylindole (DAPI) staining (final concentration 0.1  $\mu\text{g mL}^{-1}$ ) and filtered onto black polycarbonate membranes (Millipore, 0.22  $\mu\text{m}$ ) (Millipore, Billerica, MA, USA). DAPI stained bacteria subsamples were enumerated under epifluorescence microscopy (filter set for Zeiss Axioplan - G 365/FT395 LP 420; Carl Zeiss, Oberkochen, Germany) and picocyanobacteria were distinguished and enumerated under blue excitation, producing a specific yellow autofluorescence of autotrophic bacterial cells (filter set for Zeiss Axioplan-BP 450-490/FT 510/LP 520; Carl Zeiss). An image analysis software (Image-Pro Plus 5.1; Media Cybernetics, Rockville, MD, USA) was used to distinguish between cocci and elongated bacteria (bacilli and filamentous bacteria) and to estimate their mean cell volume. The total bacterial volume was obtained multiplying total bacterial abundance by the mean bacterial biovolume weighted with the bacilli/cocci ratio. According to Posch *et al.* (2001), the mean cell volumes were converted into carbon biomass with the allometric relationship between cell volume (CV) and carbon content (CC),  $\text{CC}=218\times\text{V}^{0.86}$  (in  $\text{fg cell}^{-1}$ ) (Loferer-Krößbacher *et al.*, 1998). The total bacterial CC ( $\text{mg m}^{-3}$ ) was estimated by multiplying the mean carbon biomass per cell for the bacterial density, averaged over the three sampling depths. Other methodological specifications are summarised in Straškrabová *et al.* (1999b).

### Phytoplankton

A 500 mL depth-integrated sample (sampling depth at 3 m intervals in NIV and at 2 m intervals in TRS, from the surface to the bottom) was taken at the point of maximum depth with a horizontal Van Dorn bottle and stored in polyethylenterephthalate (PET) bottles. Samples were preserved by Lugol (Vollenweider, 1974) and periodically checked for colour (Lugol added if necessary). Samples were pre-sedimented in 500 mL cylinders and concentrated to 1/10 or 2/10 of the original volume by siphoning the supernatant liquid. Counting and measuring were carried out in sedimentation chambers (Vollenweider, 1974) using an inverted microscope, equipped with phase contrast. Individual cells were counted and identified at species level, whenever possible. Cell dimensions were measured using an electronic caliper connected to a PC (Legner and Sprules, 1993) and a mean biovolume of each species was estimated from linear measures and using the closest geometrical shape (Hillebrand *et al.*, 1999). This cell volume was the parameter used for allocating a taxon to a size class. Our size classes were defined as  $\leq 0.5 \mu\text{m}^3$ , followed by 0.5–1, 1–2, 2–4  $\mu\text{m}^3$ , *etc.*, up to the largest phytoplankton cell. Phytoplankton taxa were aggregated in functional groups, following the approach proposed by Kruk *et al.* (2010), who outlined 7 morphologically based functional groups (MBFGs), separating the organisms according to size, pres-

ence of flagella, mucilaginous envelope and siliceous exoskeletons. The CC of phytoplankton was estimated from biovolume (BV) using the single conversion factor of 0.2 for all phytoplankters,  $CC=V \times 0.2$  (Straškrabová *et al.*, 1999b). Results are expressed as abundance (cells mL<sup>-1</sup>), BV (mm<sup>3</sup> m<sup>-3</sup>) and organic carbon (mg m<sup>-3</sup>).

### Zooplankton

Zooplankton samples were collected at the deepest point of each lake by taking vertical tows from the bottom to the surface with a conical plankton net (40 cm diameter, 48 µm mesh). Samples were fixed in 4% formalin directly in the field. Zooplankton was identified at species level, but coarse taxonomic levels, indicating a group of species (gr.) have been used instead of the species name for those organisms with an uncertain taxonomy (*e.g.*, *Daphnia longispina* and *Daphnia rosea* have been lumped in the *longispina* group) or when the specific identification was not possible because of morphological deformations due to the preservation medium. Counting and measuring were carried out on 4-5 subsamples following Edmondson and Winberg (1971) in a closed counting chamber under a binocular dissecting microscope at 40x. Results are expressed as abundance (individuals m<sup>-3</sup>) and biomass (mg m<sup>-3</sup>). For each sample, the length of up to 5000 individuals was measured with a calibrated eye-piece micrometer (Edmondson and Winberg, 1971) to obtain information on the zooplankton community size spectra. We usually estimated the size spectra for each sample by measuring the length of the first 300 organisms in the sample. However, for some samples, the number of measured individuals could vary from a few tens, in case of very low zooplankton densities, up to 5000 when rotifer species in the sample were dominant and only few individuals of the larger zooplankton species were present. In the latter case we increased the number of measured individuals in order to complete the entire size spectrum including the largest size classes. Length measurements were used to estimate the dry biomass per cubic meter for each zooplankton taxa, following the regression equations proposed by Dumont *et al.* (1975), Ruttner-Kolisko (1977), Shindler and Noven (1971), and Pace and Orcutt (1981). The CC of zooplankton was estimated from dry biomass (B) using a conversion factor of 0.5 for all zooplankton species,  $CC=B \times 0.5$  (Straškrabová *et al.*, 1999b).

## RESULTS

### Physical and chemical properties

The ice-free season lasted for less than 4 months in both lakes (from 5-7 July to 21-23 October in NIV and from 1-3 July to the end of October in TRS). An inverse thermal stratification was present in NIV at the time of the first measurement, when the lake was still partly covered with

ice. From 3 August to 2 September a stable thermal stratification developed, with the depth of the thermocline increasing with time (Fig. 2). By contrast, TRS did not develop a stable thermal stratification (Fig. 2). For both lakes there was a marked cooling on 17 and 18 September, associated with a snowfall event on 16 September (38 cm of snow recorded) and abundant rainfall later. This meteorological event destroyed the thermal stratification of NIV leading to a fully mixed water column.

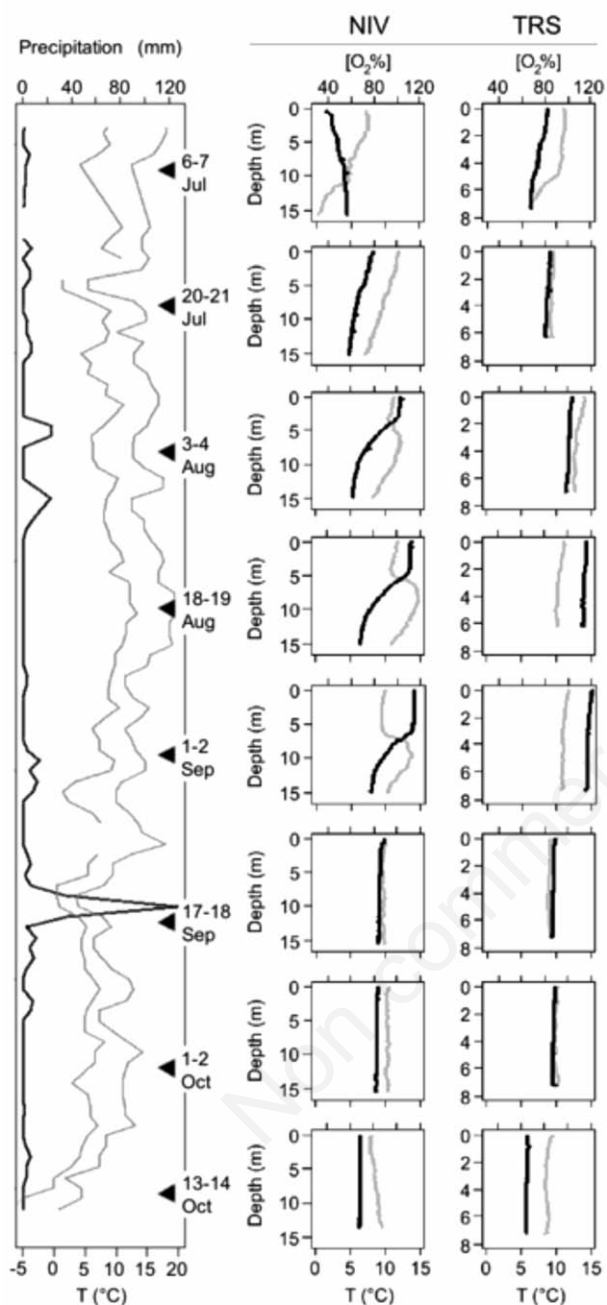
In NIV, at the time of the first measurement, winter oxygen depletion still persisted in the deepest part of the lake, but oxygen saturation increased during the season developing a maximum immediately below the thermocline in August. Lake TRS had a constant profile of dissolved oxygen, confirming its fully mixed vertical structure (Fig. 2).

Precipitation hydrochemistry is summarised in Tab. 1 and lakes hydrochemistry is summarised in Fig. 3 and Supplementary Tab. 1. The lakes studied are not sensitive to acidification processes and atmospheric input of acid compounds (see pH, alkalinity and NO<sub>3</sub><sup>2-</sup> values, Fig. 3). In fact, the ionic content conductivity was low in both lakes, but TRS was characterised by a markedly higher content of calcium, magnesium and sulphate than NIV (Fig. 3). Atmospheric deposition in 2009 at La Thuile showed markedly lower average values of pH, SO<sub>4</sub><sup>2-</sup>, total alkalinity and conductivity with respect to lake water (Tab. 1).

Nutrient concentrations (TP, ON, NO<sub>3</sub><sup>2-</sup>, TOC) in the lakes were very low. Based on TP concentrations, NIV is oligotrophic and TRS is ultraoligotrophic. A peak in the concentration of TP was observed in NIV at the time of the first measurement (20 µg P L<sup>-1</sup>), together with a high concentration of ammonium. Total nitrogen was partially accounted by IN: 36% (range: 24-53) of TN in NIV and 38% (range: 14-73) of TN in TRS. The average TN and

**Tab. 1.** Precipitation amount and chemical composition of the atmospheric deposition collected at the sampling station of La Thuile (Aosta, Italy). Chemical data are volume-weighted averages over 23 weekly samples collected in 2009.

Annual precipitation (mm)	1640
pH	5.62
Alkalinity (meq L <sup>-1</sup> )	0.03
Conductivity (µS cm <sup>-1</sup> ) (20°C)	9.36
Ca <sup>2+</sup> (mg L <sup>-1</sup> )	0.56
Mg <sup>2+</sup> (mg L <sup>-1</sup> )	0.05
Na <sup>+</sup> (mg L <sup>-1</sup> )	0.30
K <sup>+</sup> (mg L <sup>-1</sup> )	0.22
NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	0.30
SO <sub>4</sub> <sup>2-</sup> (mg L <sup>-1</sup> )	0.63
NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.25
Cl <sup>-</sup> (mg L <sup>-1</sup> )	0.36
TN (mg L <sup>-1</sup> )	0.88
ON (mg L <sup>-1</sup> )	0.32
DOC (mg L <sup>-1</sup> )	1.08



**Fig. 2.** Left panel: meteorological data measured at the weather station of lake Serrù (45° 27' 33.13" N, 7° 7' 32.86" E, 2275 m asl) showing daily precipitation (black line) and maximum and minimum daily temperature (grey lines) measured in the period 1 July–15 October 2009; triangles indicate the dates of the measurements in lake Nivolet *superiore* (NIV) and Trebecchi *superiore* (TRS) (Gran Paradiso National Park). Right panels: temperature (black) and dissolved oxygen (grey) profiles in NIV and TRS for each measurement date.

IN concentrations in the atmospheric deposition in 2009 were higher than in the lakes (Tab. 1) and IN in atmospheric deposition accounted for 62.5% of TN. Mean TN:TP ratios were 25 (range: 11–32) in NIV and 35 (range: 26–110) in TRS, corresponding to a mean molar ratio of 56 (range: 24–70) in NIV and 78 (range: 58–244) in TRS. Silicon is a nutrient for diatom algae and its concentration was higher in TRS than in NIV; after the peak at the beginning of the sampling period, silicon values decreased in both lakes.

### Pico-prokaryotes

Pico-prokaryotes were composed of bacteria (we did not differentiate between *Bacteria* and *Archaea*) and picocyanobacteria. Bacteria abundance differs in the two lakes, reaching almost  $3 \times 10^6$  cells mL<sup>-1</sup> in NIV and  $1.3 \times 10^6$  cells mL<sup>-1</sup> in TRS (Fig. 4; Supplementary Tab. 1). Conversely, their fluctuations during the season were very similar, showing a peak of abundance at the end of August. In NIV the highest numbers have been found near the bottom, whereas homogeneous densities were recorded in the water column of TRS. Immediately after melting (in July) the bacteria number was high in deeper layers, but decreased abruptly in the following two weeks. This pattern is even more evident looking at the biovolume data, particularly in NIV. The mean cell biovolumes were similar in the two lakes, ranging from 0.1 to 0.3  $\mu\text{m}^3$  cell<sup>-1</sup>. Larger cells were found near the bottom in NIV and in the middle of the water column in TRS. Smaller cells were recorded at the peak of abundance during late summer.

Picocyanobacteria abundance markedly differed in the two lakes (data reported in Supplementary Tab. 1), with an higher peak in NIV ( $61 \times 10^3$  cells mL<sup>-1</sup>, August 3) than in TRS ( $4.6 \times 10^3$  cells mL<sup>-1</sup>, July 7). As a general spatial pattern, their number increased near the bottom and was very low near the surface.

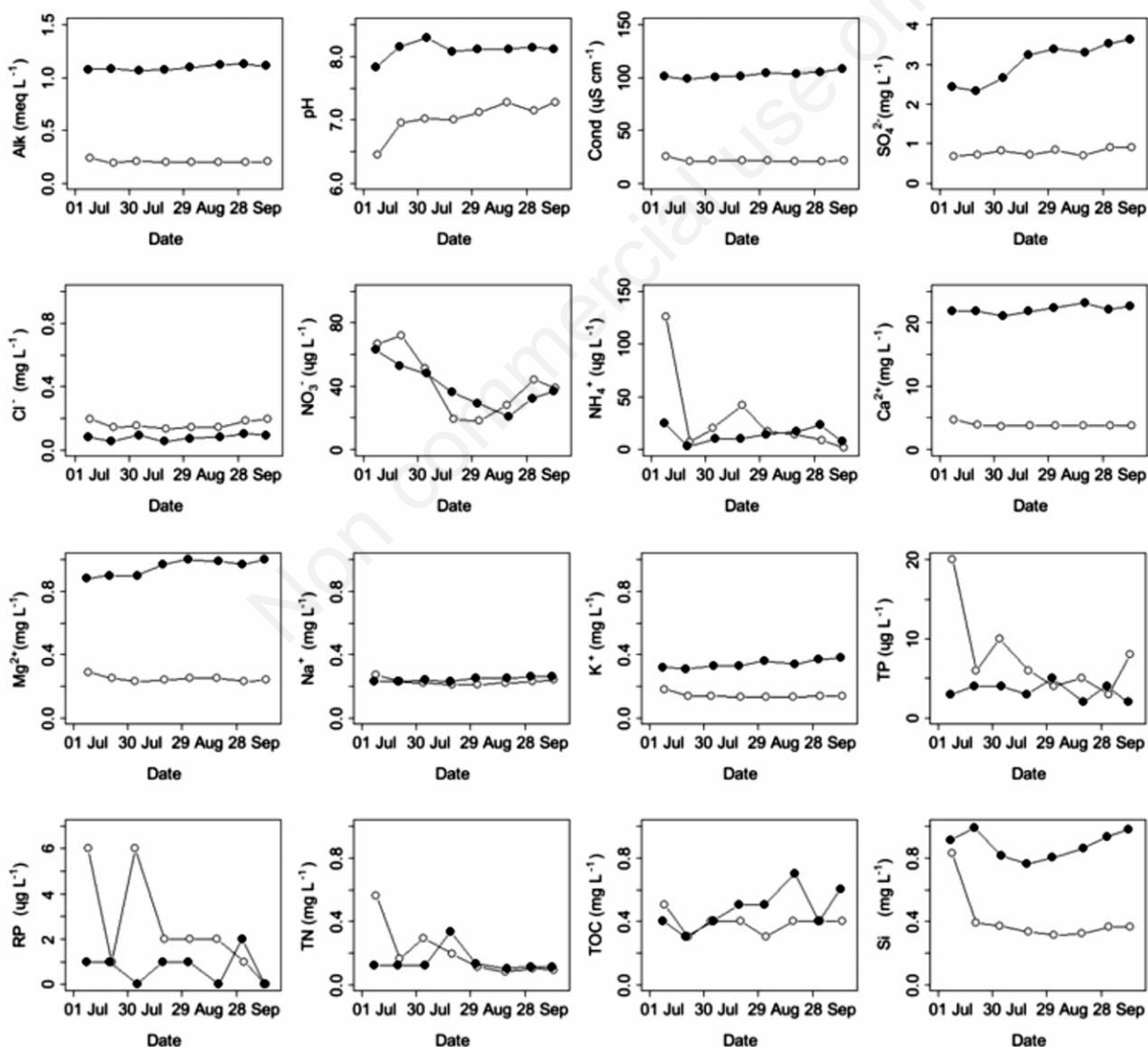
### Phytoplankton

Looking at the seasonal dynamics (Fig. 5), a bimodal pattern is evident for both lakes, with the first peak immediately after the disappearance of the ice cover and the second one occurring at the end of the summer period. In terms of densities, for NIV the first peak is lower than the second. However, in terms of biomass the opposite is true (Fig. 5). This is due to the fact that the second bloom is mainly due to flagellates, while in the first bloom some large diatoms have also been detected. In TRS there are three distinct peaks of abundance, whereas a bimodal pattern is visible in the biomass record. Concerning the biovolume variability of the whole phytoplankton assemblages, NIV showed higher fluctuations, both between and within samples. The common occurrence, in NIV samples, of taxa belonging to different size classes explains a higher variability of the bio-

volume range recorded in this lake. A raw comparison between the two lakes seems to suggest slightly lower average cell sizes in TRS: this is mainly due to the dominance in NIV of some large taxa, such as *Peridinium* spp., *Cryptomonas* spp. and *Surirella* sp., absent in TRS.

Fig. 6 shows the seasonal changes of phytoplankton in terms of MBFG. Starting from biomass data, the summer samples in NIV were dominated by groups V (unicellular flagellates of medium to large size) and VI (all diatom taxa), whereas, in TRS, groups VI was dominant. Autumn samples were characterised by groups VII (large mucilaginous colonies, in this case the chlorophyte *Willea irregularis* Schmidle) and VI in NIV and by groups V (including *Cryp-*

*tomonas* spp. and *Plagioselmis nannoplantica* Skuja) and VI in TRS. Considering the abundance of data, the role of smaller taxa is emphasised in summer samples: groups I (small organisms with high surface-to-volume ratio, mainly *Pseudanabaena* sp. in this case) and II (small chrysophytes) are the most important in NIV, whereas in TRS group II is clearly dominant (with *Kephyrion* sp. and *Chromulina* sp.), followed by group VI (small *Cyclotella* spp.). In autumn samples, smaller taxa declined: in NIV, group VII and the medium sized group IV are dominating, due to *Willea irregularis*, together with *Aphanocapsa* sp. and *Sphaerocystis schroeterii* Chodat. In TRS, group V was the dominant in September and October (mainly *Plagioselmis nannoplanc-*



**Fig. 3.** Summer dynamics of the main chemical variables in lakes Nivolet *superiore* (NIV) and Trebecchi *superiore* (TRS) (Gran Paradiso National Park). Open circles refer to NIV and black circles to TRS.

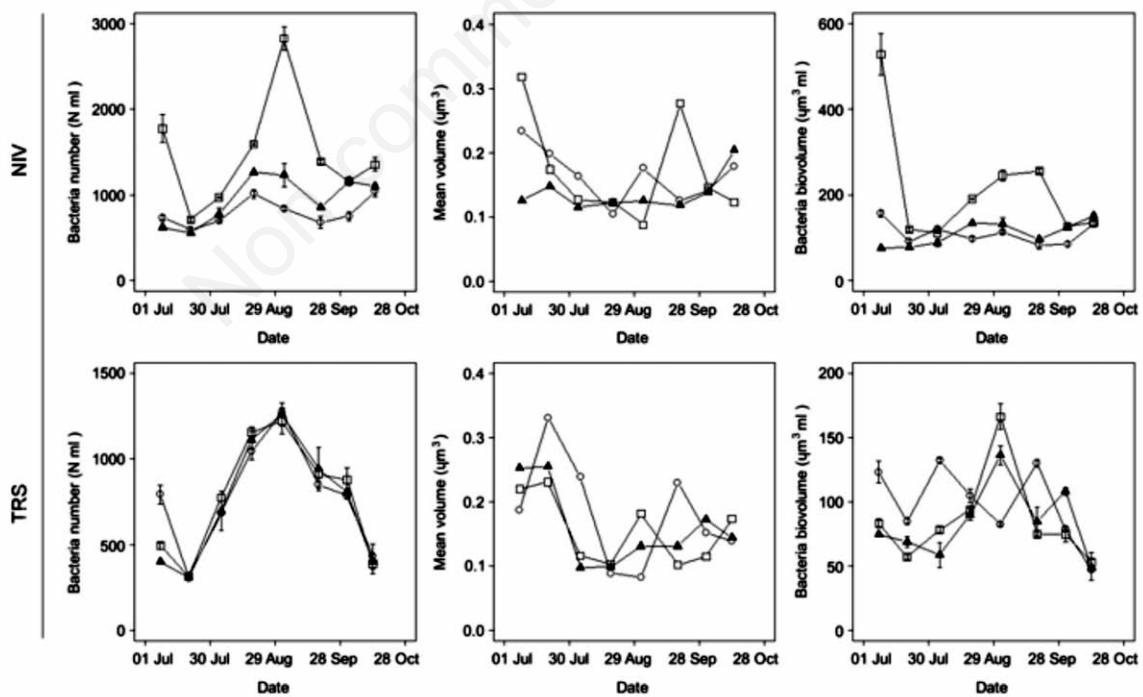
*tica*), together with group VI. Comparing the two lakes, a different pattern emerged in summer-to-autumn transition: in NIV a shift from flagellates (both small and large) to colonial and mucilaginous forms occurred, while in TRS the flagellates always dominated, with smaller ones (group II) most important in summer and larger ones (group V) most important in autumn. The dynamic of diatoms, though always being important in both lakes in terms of biomass and sometimes abundance in TRS, did not show a clear seasonality. However, we should consider that their presence in the pelagic samples could be reasonably due to a recruitment from the bottom of the lakes, due to re-suspension.

### Zooplankton

The zooplankton communities consisted of rotifers, copepods and cladocerans. A complete list of the species with their densities is reported in Supplementary Tab. 1. Ten different zooplankton taxa were recorded in NIV and 13 in TRS. The composition of the zooplankton communities was very similar in both lakes, sharing the same dominant species. The most common Crustaceans were *Arctodiaptomus alpinus* Imhof, *Cyclops* gr. *abyssorum* Sars and *Daphnia* gr. *longispina* Müller, which were

found in all 8 samples in both lakes. The crustacean community was completed by *Eucyclops serrulatus* Fisher (both in NIV and TRS), *Daphnia* gr. *pulicaria* Forbes (both in NIV and TRS, previously considered as Alpine populations of *Daphnia middendorffiana* Fisher; Tiberti, 2011), *Alona quadrangularis* Müller (only in TRS) and *Chydorus sphaericus* Müller (only in TRS). Concerning the rotifer community, *Keratella quadrata* Müller was by far the most common species in both lakes.

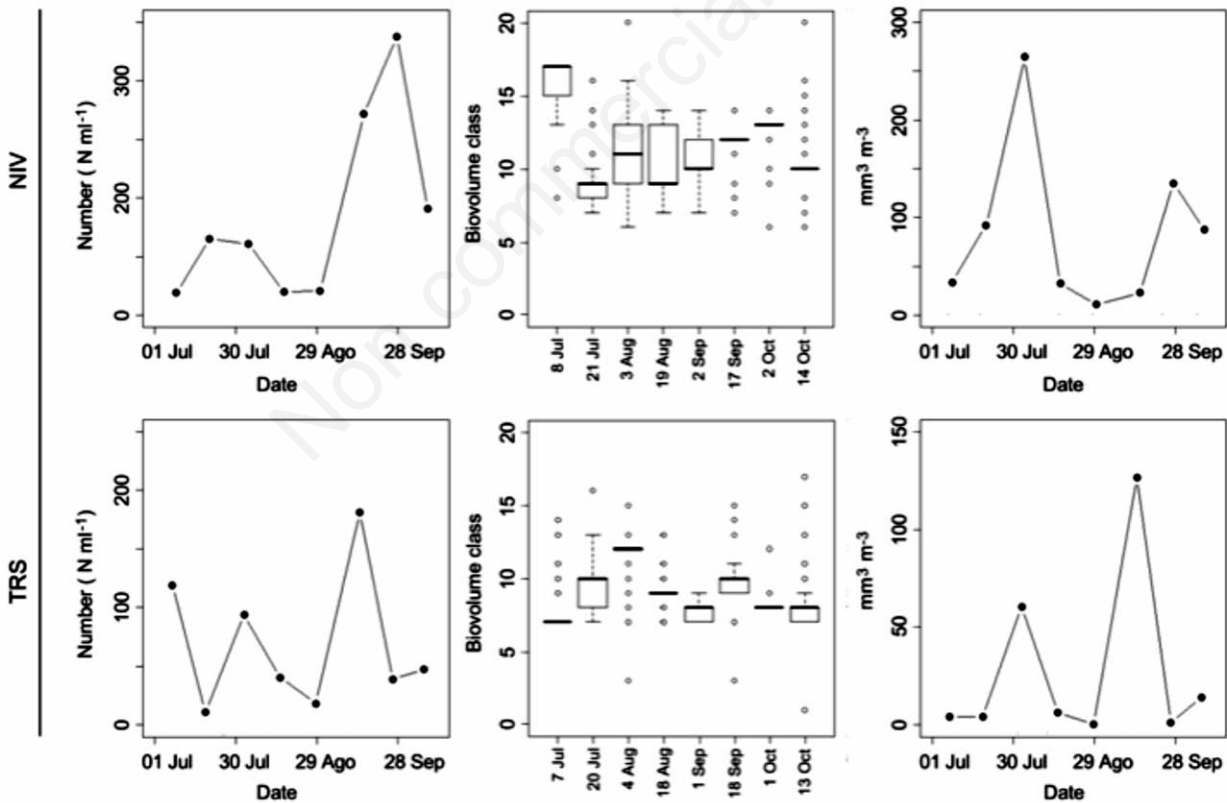
Fig. 7 describes the zooplankton dynamics in NIV and TRS, which showed the same patterns in the two lakes. Lake Trebecchi *superiore* supported higher zooplankton densities and biomass than NIV. The total number of individuals per cubic meter showed two peaks, the first one being determined by rotifers at the beginning of the summer and the second mainly determined by *Daphnia* gr. *longispina* in September. The peak of copepods was observed slightly earlier than that of cladocera. On the contrary, there was only one peak of zooplankton biomass in September, which was sustained mainly by crustaceans, since the contribution of rotifers to the total biomass was always very small. The size spectra of the zooplankton community grew in the course of summer and reached relatively stable values in midsummer.



**Fig. 4.** Dynamics of the abundances [ $\pm 1$  standard error (se)], mean cell volumes and total biovolumes ( $\pm 1$  se) of the bacterial community at three sampling depths in lakes Nivolet *superiore* (NIV) and Trebecchi *superiore* (TRS) (Gran Paradiso National Park). Open circles refer to surface samples, black triangles to samples collected in the middle of the water column (8.5 m in NIV and 3.7 m in TRS) and open squares to samples collected close to the bottom (15.5-16.5 m in NIV and 7.0-7.2 m in TRS).

Detailed ecological dynamics could be described only for the most common zooplankton species. *Cyclops* gr. *abyssorum* reached its maximum density at the end of August (Supplementary Tab. 1). The population at the beginning of the summer was mainly composed by nauplii and copepodids, but some large overwintering reproductive stages, including some adult males and egg-carrying females, were still present in both lakes. The reproductive stages disappeared from the second sampling (20-21 July) and reappeared from mid-August until October in NIV and just in mid-August in TRS. Naupliar stages of *Cyclops* were found during the whole measurement period. *Arctodiaptomus alpinus* reached its maximum density on 4 August in TRS and on 2 September in NIV (Supplementary Tab. 1). On the first sampling date, the population was composed exclusively of nauplii, which tended to completely disappear later in the season. The reproductive stages of *Arctodiaptomus alpinus* were found from the beginning of August till the end of the summer, both in NIV and TRS. *Daphnia* gr. *longispina* reached its maximum density on 17

September in NIV and on 1 October in TRS (Supplementary Tab. 1). At the beginning of summer there were only small individuals, while the first reproductive stages were found starting from August, both in NIV and TRS. On 17 and 18 September there were the first cases of resting eggs (ephippia) production, which continued and intensified until the end of summer. *Daphnia* gr. *pulicaria* was found at low density levels, reaching higher densities in late August and early September (Supplementary Tab. 1). No males have been found during the sampling campaign, in agreement with the hypothesis that those populations are obligatory parthenogenetic (Tiberti, 2011). Interestingly, both *Daphnia* gr. *longispina* and *Daphnia* gr. *pulicaria* showed a dark cuticular pigmentation. Concerning rotifer species, the dynamics of *Keratella quadrata* is substantially the same as the dynamics of the entire rotifer community, reaching its maximum density and biomass at the first sampling date (Supplementary Tab. 1). *Polyarthra* gr. *dolichoptera* Ehrenberg appeared at the end of the season, only in NIV (Supplementary Tab. 1).



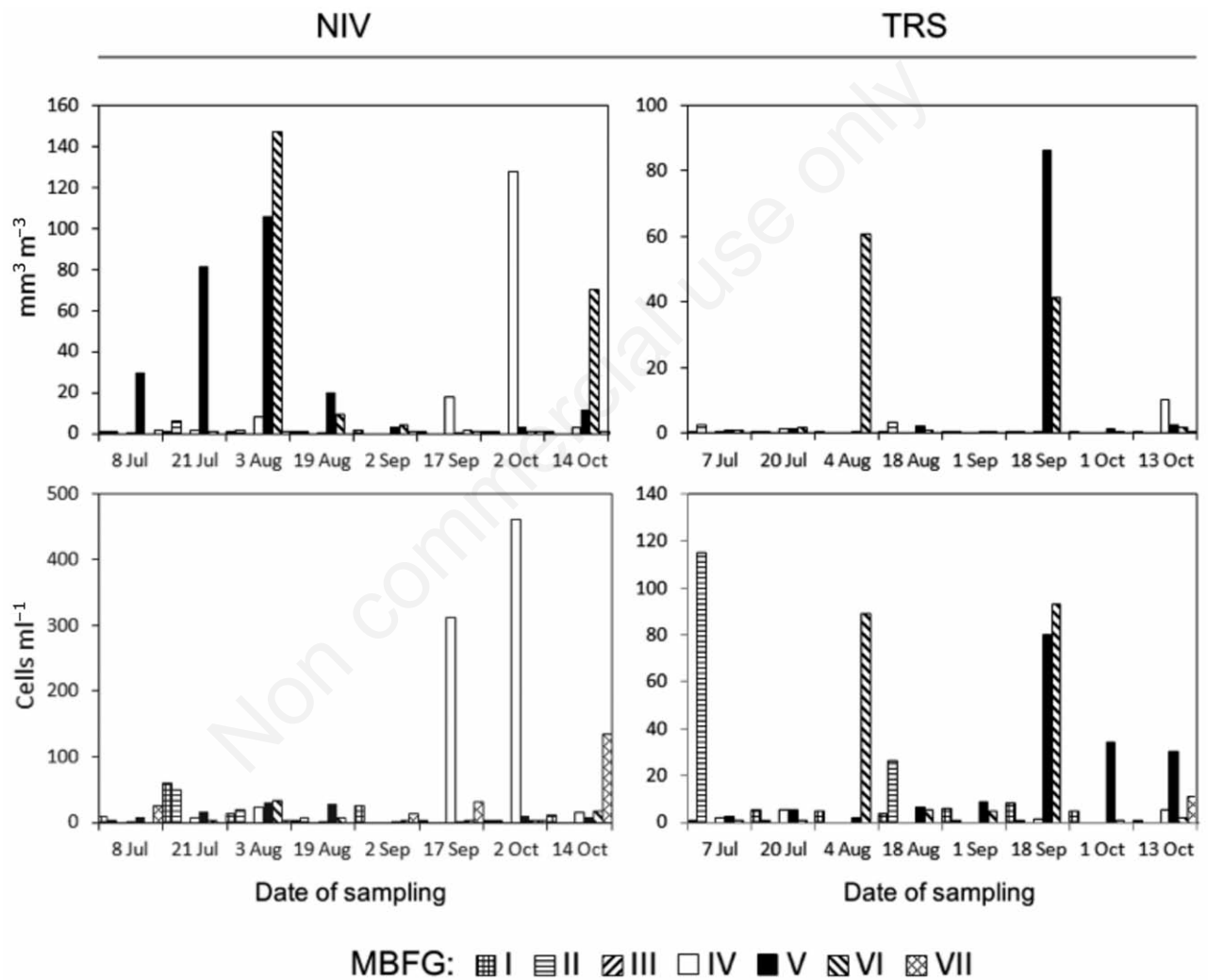
**Fig. 5.** Phytoplankton community dynamics within the lakes Nivolet *superiore* (NIV) and Trebecchi *superiore* (TRS) (Gran Paradiso National Park). Left panels: phytoplankton densities. Centre panels: size variation within the phytoplankton community based on 20 size classes; the box-plots show the median and the 1<sup>st</sup> and 3<sup>rd</sup> quartiles; notches indicate  $\pm 1.58$  multiplied by the interquartile range and divided by the square root of the total number of measured specimens; empty circles are outliers. Right panels: phytoplankton biomass.

## DISCUSSION

### Hydrochemical characteristics

The ecosystems studied are naturally oligotrophic or ultraoligotrophic and, according to the Redfield's ratio, the high TN:TP molar ratios suggest phosphorus limitation of the algal growth (O'Sullivan and Reynolds, 2004). During summer 2009, excluding the unusually high TP concentration at the time of the first measurement in NIV,

we could not find phosphorus or nutrient input events due to pasture, tourism or other human activities and we can reasonably exclude anthropogenic nutrient enrichment. The phosphorus peak observed in NIV during the first measurement could be explained by a release from the sediment owing to the anoxia of the deeper layers, as demonstrated by the oxygen profile and by the typical smell of the water. As observed by Ohlendorf *et al.* (2000), Alpine lakes can be naturally affected by anoxic



**Fig. 6.** Seasonal fluctuations of the phytoplanktonic morphologically-based functional groups (MBFG; Kruk *et al.*, 2010) within lakes Nivolet *superiore* (NIV) and Trebecchi *superiore* (TRS) (Gran Paradiso National Park): abundance data in the upper panels, biomass data in the lower ones. Group I: small organisms with high surface:volume ratio (Chlorococcales, Chroococcales, Oscillatoriales, Xanthophyceae, Ulothricales); Group II: small flagellated organisms with siliceous exoskeletal structures (Chrysophyceae); Group III: large filaments with aerotopes (Nostocales, Oscillatoriales); Group IV: organisms of medium size lacking specialized traits (Chlorococcales, Oscillatoriales, Xanthophyceae, Zygnematophyceae); Group V: unicellular flagellates of medium to large size (Cryptophyceae, Dinophyceae, Euglenophyceae, Volvocales, Chlorococcales); Group VI: non-flagellated organisms with siliceous exoskeletons (Bacillariophyceae); Group VII: Large mucilaginous colonies (Chlorococcales, Chroococcales, Oscillatoriales).

conditions at the sediment-water interface during winter ice-covered conditions and particularly during thawing. Bottom oxygen depletion usually persists until the lake is completely ice-free and, subsequently, oxygen undergoes a rapid increase. In the absence of inputs of glacial silt, the scarcity of nutrients allows for very transparent waters: in Alpine lakes most of the organic carbon is dissolved and TOC concentration is a good indicator of water transparency to visible and UV radiations (Tiberti and Iacobuzio, 2013). The measured low values of TOC indicate that the lakes under study are naturally transparent, as also demonstrated by the dark pigmentation of both *Daphnia* gr. *longispina* and *Daphnia* gr. *pulicaria*, which are known to develop cuticular pigmentation as a defence against UV radiations (Hansson and Hylander, 2009).

Both NIV and TRS show a clear connection between lithology of the watershed and lake hydrochemistry. The basin of TRS is dominated by highly soluble calcareous schists (Compagnoni *et al.*, 1974), which through weathering processes, markedly increase pH and alkalinity. Conversely, NIV, whose basin is totally dominated by insoluble acidic gneiss, has a low weathering rate. However, despite the composition of its watershed, according to Turner *et al.* (1986) and Mills and Schindler (1986) NIV is not affected by acidification, as demonstrated by pH and alkalinity with minimum values of 6.45 and 196  $\mu\text{eq L}^{-1}$ , respectively.

Ion composition of both lakes is also influenced by the inputs from the basin: calcium and bicarbonate from weathering processes were always the prevailing ions, followed by  $\text{SO}_4^{2-}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^+$  (in NIV) or  $\text{K}^+$  (in TRS). The  $\text{SO}_4^{2-}$  to  $\text{Cl}^-$  ratio in lake water (3.6-5.9 in NIV, 29.6-64.8 in TRS) proved to be markedly higher than the corresponding ratio in seawater (0.103).

High  $\text{SO}_4^{2-}$  concentrations in lake water can be a symptom of long-range transport of air-borne pollutants. This is not the case for NIV and TRS, as the content of sulphate in atmospheric depositions (Tab. 1) was markedly lower than in lakes. As a consequence, the  $\text{SO}_4^{2-}$  content in lake water can be largely attributed to weathering of rocks and soils bearing sulphur minerals. This was confirmed also by higher concentrations of  $\text{SO}_4^{2-}$  in TRS, whose catchment is formed by more soluble rocks with respect to NIV. Also, the  $\text{Na}^+$  to  $\text{Cl}^-$  ratio in lake water (2.65-4.60 in TRS, 1.26-1.64 in NIV) was above the ratio in marine water (0.86) and in precipitations at La Thuile (0.82), indicating a weathering contribution to  $\text{Na}^+$  concentration (Rogora *et al.*, 2008).

Concentrations of nitrogen (N) compounds can be used as an index of the impact of human settlement on remote lakes: when lakes are subject to low N input ( $<1 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ), nitrate ( $\text{NO}_3^-$ ) concentrations are usually low and ON accounts for a substantial proportion of TN (Hedin *et al.*, 1995; Kaushal and Lewis, 2003; Rogora

*et al.*, 2008). Long-range transport of N compounds from intensively cultivated or industrial regions affect lakes in different remote areas of Europe (Allott *et al.*, 1995; Kopáček *et al.*, 2001; Evans *et al.*, 2001; Wright *et al.*, 2005). Lake Nivolet *superiore* and TRS could be threatened by the proximity of the Po plain (Northern Italy), one of the most densely populated and industrialised regions in Europe. Indeed, the areas surrounding the Alpine rim produce nitrogen deposition among the highest in Europe (Rogora *et al.*, 2001) and, in 2009, the total N concentration ( $0.88 \text{ mg L}^{-1}$ ) and deposition rates ( $14.4 \text{ kg N ha}^{-1} \text{ y}^{-1}$ , calculated by annual precipitation and average N content) at the sampling station of La Thuile were high and similar to those usually reported for the Alpine range (N deposition rate:  $14\text{-}15 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ; Rogora *et al.*, 2006). However, the final nitrogen concentration in the lakes depends on retention process (closely related to vegetation and catchment characteristics) (Marchetto *et al.*, 1995; Rzychon and Worsztynowicz, 2008), in addition to N deposition rate. Inorganic nitrogen concentration in the lakes studied ( $0.07 \text{ mg N L}^{-1}$  in NIV, and  $0.04 \text{ mg N L}^{-1}$  in TRS) was considerably lower than the values found in other lakes in the Gran Paradiso National Park ( $0.17 \text{ mg L}^{-1}$ , average IN concentration from a set of 15 Alpine lakes sampled in 2009, unpublished data) and for the Alps ( $0.8\text{-}1.0 \text{ mg L}^{-1}$ ) (Rogora *et al.*, 2003, 2008), and it was comparable with the values from European and non-European pristine areas subject to low atmospheric input of N compounds (Boavida and Gliwicz, 1996; Tartari *et al.*, 1998; Murphy *et al.*, 2010). Moreover, the dominance of ON on IN suggests that retention processes at these sites were able to mitigate the effects of the high N deposition. This is not necessarily an indication that a weak impact of long range pollutants and some confirmatory measurements (*e.g.*, trace metals and organochlorine compounds concentrations) would be required, but the lakes studied are probably less affected by N and deposition of pollutants than other Alpine lakes (Tiberti *et al.*, 2010), possibly due to a higher retention capacity and to a lower amount of precipitation in the Gran Paradiso National Park area (Frei and Schär, 1998).

### Trophic compartements in Alpine lakes

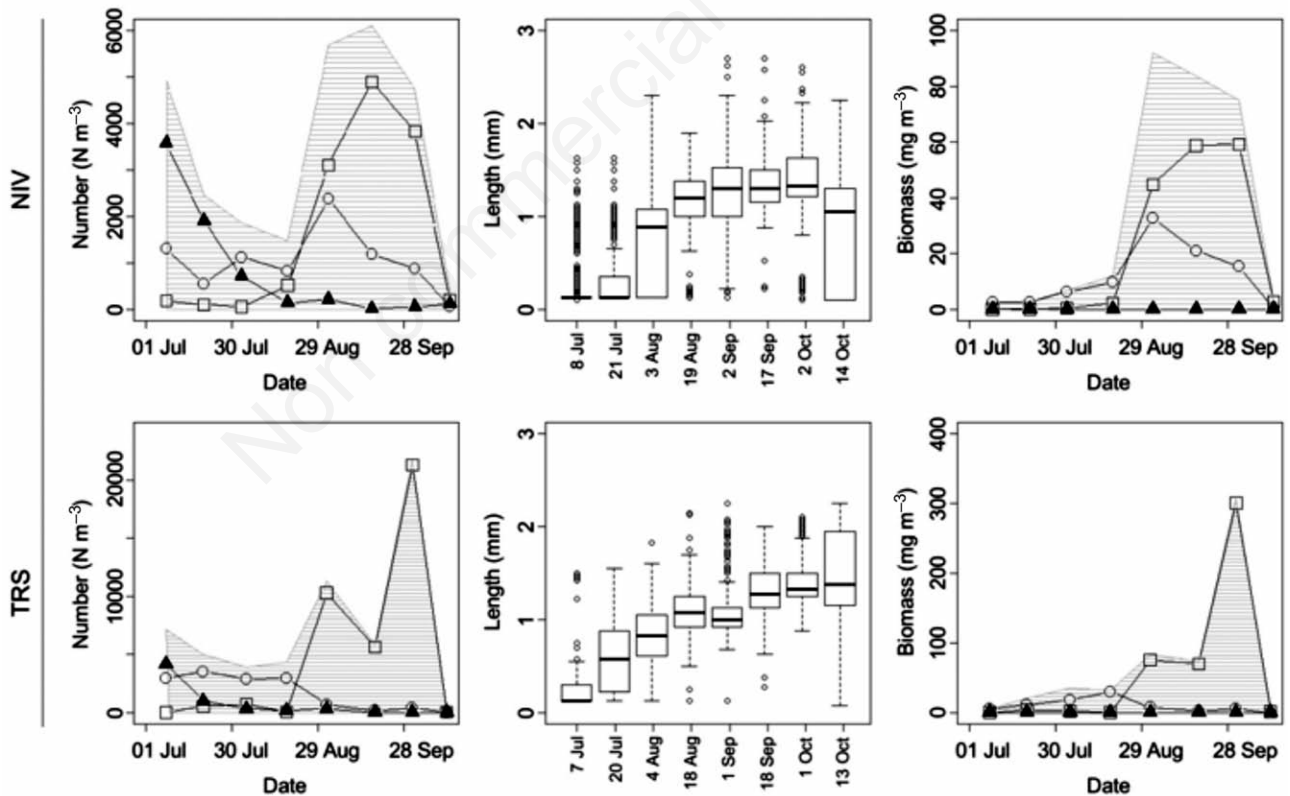
The lakes under study are exposed to extreme climatic conditions causing a short ice-free season and rapid changes in their physical properties. Temperature profiles change under the influence of seasonal, geographic, morphometric and climatic factors: despite its higher altitude, owing to its better exposition and its moderate depth, TRS showed a polymictic regime and was slightly warmer than NIV, which instead showed a clear dimictic regime. Dissolved oxygen profiles are strongly influenced by thermal stratification. Except for the first sampling date, TRS always showed a constant oxygenation along the water col-

umn, confirming its fully mixed vertical structure. At the beginning of the ice-free season (on 8 July), the winter oxygen depletion still persisted at the bottom of NIV and a maximum of oxygen, immediately below the thermocline, was present during the summer stratification (from 3 August to 2 September). It is well known that the thermocline acts as a barrier for vertical algal movements, resulting in high algal densities and correspondingly high oxygen production (Hansson, 1996).

Looking at the pico-prokaryote abundance, the two lakes showed a similar seasonal distribution of bacterial numbers, with a very clear bell-shaped pattern peaking at the end of August, but different densities. In NIV, the number of bacteria was higher in comparison to other Alpine lakes and to TRS, possibly due to different RP concentrations and to a greater depth of NIV, which could offer more hospitable ecological niches. In lake Paione superiore (Central Italian Alps), the range of bacteria abundance was  $0.1\text{-}1 \times 10^6$  cells  $\text{mL}^{-1}$  (Callieri and Bertoni,

1999), in Jöri lake (Eastern Swiss Alps) it was  $0.7\text{-}1.7 \times 10^6$  cells  $\text{mL}^{-1}$  (Hinder *et al.*, 1999), and in Gossenköllesee (Tyrol, Austria) it was even lower ( $2.5\text{-}5.5 \times 10^5$  cells  $\text{mL}^{-1}$ ) (Wille *et al.*, 1999).

In both lakes, the bacterial density reached its maximum between the two peaks of phytoplankton and zooplankton in a period of P-limitation. Bacteria are known to outcompete phytoplankton for P (Vadstein, 2000) and could have taken advantage from the organic carbon excretion during the first phytoplankton peak and the subsequent cellular degradation during the population decrease (Callieri *et al.*, 2006). At the beginning of the season, NIV showed a high peak of bacterial numbers and biomass, in correspondence with the TP and RP peaks, near the bottom. This could be due to anaerobic ammonium-oxidising (anammox) bacteria, which are mainly *Planctomycetes* and able to grow anaerobically oxidising ammonia (Madigan *et al.*, 2009). Near the bottom of NIV, we found oxygen depletion and an ammonia peak; these



**Fig. 7.** Zooplankton community dynamics within the lakes Nivolet superiore (NIV) and Trebecchi superiore (TRS) (Gran Paradiso National Park). Left panels: zooplankton densities. Centre panels: community size spectra; the box-plots show the median and the 1<sup>st</sup> and 3<sup>rd</sup> quartiles; notches indicate  $\pm 1.58$  multiplied by the interquartile range and divided by the square root of the total number of measured specimens; open circles are outliers. Right panels: zooplankton biomass. Shaded areas: total density (left panels) or biomass (right panels); black triangles: rotifers; open circles: copepods; open squares: cladocerans.

conditions strengthen the hypothesis of *Planktomyces* (large bacteria) which are likely to occur in marine and freshwater habitats.

As for the bacterial size, we noted larger cells at the beginning of the ice-free period at low grazing pressure, when zooplankton and flagellates were low. The mean cell size decreased later in the season, in correspondence to the increase of larger cell consumers. We cannot exclude that the peak of larger cells on 17 September was spurious and related to a strong precipitation event, registered just before that date, and the subsequent runoff carrying terrestrial bacterial species into the lakes.

The other component of pico-prokaryotes, the picocyanobacteria, in general are of low importance in high altitude lakes for their vulnerability to UVR and photosynthetic active radiations (PAR) of high intensity (Callieri *et al.*, 2001). Nevertheless, they were numerous in NIV, near the bottom, as compared with other Alpine lakes (Callieri and Bertoni, 1999; Wille *et al.*, 1999). This result could be explained by the neutral pH of NIV and by the maximum depth of the lakes, which constitutes in itself a potential refuge from harmful radiation.

Owing to the scarcity of nutrients (especially phosphorus) we may expect phytoplankton to be dominated by species with small cell dimensions allowing for a higher surface:volume ratio and therefore for a more efficient nutrient uptake (Callieri *et al.*, 2006). Indeed, the measurements indicated that phytoplankton community in NIV and TRS was commonly dominated by flagellates (chrysophytes, dinoflagellates, and cryptophytes), as generally reported for European Alpine lakes (Goldman and Home, 1983; Tolotti *et al.*, 2006). Flagellates are usually more resistant to harsh winter conditions typical of high mountain lakes than other phytoplankters, and they are able to thrive under ice and form inocula, giving rise to the spring growing phase soon after ice melting (Reynolds, 2006).

The summer algal succession was similar in both NIV and TRS. During the few months of the high-altitude ice-free season, three periods were evident: i) a spring development, during snowmelt, ii) a midsummer period of minimal algal abundance, and iii) a fall growth. This double-peak structure is common in Alpine lakes (Mcknight *et al.*, 1990; Winder *et al.*, 2003).

In wintertime, the primary production of Alpine lakes is constrained by abiotic conditions, such as low temperature and light scarcity, owing to the formation of a thick ice cover (Mcknight *et al.*, 1990). Only with ice thawing and with the rapid nutrient flushing during snowmelt, algae can develop (Straila, 2005; Rott, 1988) and produce the first seasonal peak. The spring algal bloom is considered to sustain the growth of zooplankton herbivores, which often control algae, reducing their density within the so called *clear water phase* (Lampert *et al.*, 1986). The same ecological mechanism could have produced the

mid-summer collapse of phytoplankton observed in NIV and TRS. The second algal blooms developed when zooplankton communities were still increasing in biomass and abundance and, at this phase, the zooplankton grazing was unable to control the phytoplankton community. As a consequence, phytoplankton shifted its taxonomic composition towards less edible taxa such as mucilaginous colonies in NIV and the medium sized flagellates in TRS. The decrease of temperatures, affecting the rates of zooplankton grazing (Vidal, 1980) is a further factor contributing to the decrease of predation pressure. At the end of summer, abiotic conditions returned to play a major role in shaping algal dynamics and the entire system plunged again towards winter conditions.

Excluding diatoms, whose dynamics was not clear and could have been affected by re-suspension from the bottom, the phytoplankton dynamics and assemblages were similar in the two lakes. The presence in NIV of poor P-competitors such as *Gymnodinium* sp. and *Peridinium* sp. (group V), on the other hand, could indicate a less severe nutrient limitation in this lake, while group II chrysophytes in TRS indicated permanent low-nutrient conditions (Kruk *et al.*, 2010). In this respect, the higher nutrient availability in NIV could support larger-sized flagellates (Litchman and Klausmeier, 2008) and, later, the observed development of little edible (Reynolds, 2007) mucilaginous colonies (group VII).

The zooplankton dynamics was almost identical in TRS and NIV, showing a transition from a community dominated by small zooplankters to a community where large particle-feeders (*e.g.*, *Daphnia* sp.) were dominant. The zooplankton ecological succession showed a few distinct stages: i) *Early zooplankton community*. Immediately after ice melting, rotifers dominated, while the crustacean community was composed of larval and juvenile stages. At this stage, the size spectrum of the entire zooplankton community was at its seasonal minimum. ii) *Transition zooplankton community*. In early summer (July) rotifers declined while crustaceans grew in number and size. The total number of individuals decreased owing to the decline of rotifers, but the total biomass showed a small increase and the community size spectrum increased. iii) *Maturity zooplankton community*. This stage was dominated by large crustacean species with the seasonal peaks of copepods and Daphnids. A strong increase of number and biomass of zooplankton was coupled with the highest community size spectrum. iv) *Senescence zooplankton community*. At this stage the zooplankton community collapsed, with a reversal of the seasonal patterns of biomass and number of individuals.

Biotic interactions among zooplankton species seemed to play a key role in determining the seasonal dynamics. Predation by *Cyclops* (Brandl, 1998, 2005) and competition with large grazers (*e.g.*, *Daphnia* sp.; Eby *et*

al., 2006) could explain the observed rotifer decline between stages i) and ii) and the fast-paced dynamics of stage iii) (Manca and Comoli, 1999). In general, during the short Alpine summer different zooplankton taxa succeeded one another and completed their life cycle before the end of the ice-free season, without a clear stationary phase in the zooplankton succession.

Occasionally, other species, usually inhabiting the littoral zone (*Eucyclops serrulatus*, *Alona quadrangularis* and *Chydorus sphaericus*) were also found in pelagic samples, probably as a result of the action of wind or of a seasonal increase of their densities, but their contribution to the community biomass was always very small.

### Non-stationary dynamics of Alpine lakes

Some differences were detected in the ecological dynamics of NIV and TRS, which can probably be ascribed to the natural variability among ecosystems or to the different morphology and hydrochemistry of NIV and TRS. However, the similarities between the two ecosystems remain strong and some of the observed processes are almost identical in NIV and TRS.

In Fig. 8 we summarise the summer succession of the two lakes from ice melting to water cooling in autumn.

**Initial stage.** At the beginning of summer, ice breakup and snowmelt cause a sudden shift from light-limited conditions to high light intensity and a large flushing rate of terrestrial nutrients. Bacteria dominate the plankton community, while the primary production is low and still af-

ected by the winter ice cover. The zooplankton community is at its seasonal minimum and is dominated by rotifers and larval stages of crustaceans, with a few large, overwintering cyclopoids and daphnids carrying eggs.

**Early plankton community.** Owing to nutrient availability, phytoplankton reaches its first peak at the expenses of bacteria, while there is a transition from rotifer to crustacean dominance in the zooplankton community.

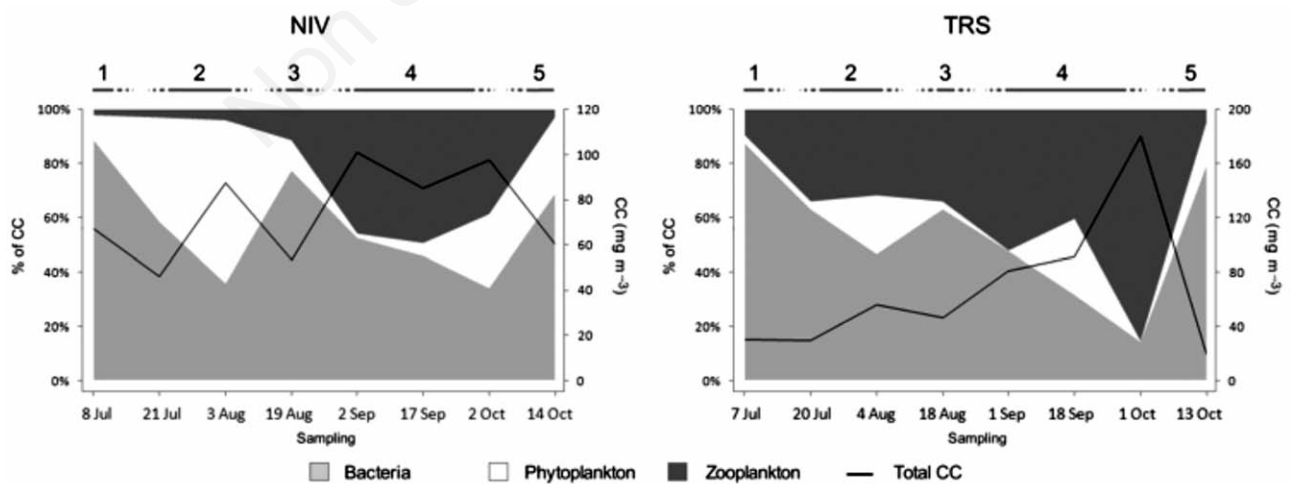
**Clear water phase.** Phytoplankton declines and bacteria return to dominate the plankton community. Zooplankton is still in transition.

**Late plankton community.** Owing to the increase of zooplankton density and biomass and the two consecutive peaks of copepods and daphnids, the total CC of the plankton community increases. Phytoplankton reaches a second peak, shifting its taxonomic composition towards less edible taxa, and bacteria reach the maximum seasonal density and begin to decline.

**Plankton community senescence.** The rapid water cooling puts an end to the summer succession, with the zooplankton community collapsing and the bacteria becoming dominant.

The first and the last stages of the ecological succession were strongly affected by climatic conditions, while in the middle of summer, when climatic conditions became less extreme, the community succession was increasingly determined by internal dynamics (e.g., competition and predation).

There are some clear similarities between this successional scheme and the PEG (plankton ecology group)



**Fig. 8.** Percentage partitioning of organic carbon within the plankton community (primary axis), total carbon content (CC) within the plankton community (secondary axis) and partitioning of the summer dynamics into 5 successional stages. 1, initial stage; 2, early plankton community; 3, clear water phase; 4, late plankton community; 5, plankton community senescence. Data collected during the 2009 ice-free summer season in two Alpine lakes, Nivolet superiore (NIV) and Trebecchi superiore (TRS) in Gran Paradiso National Park (western Italian Alps).

model (Sommer *et al.*, 1986), which describes the idealised plankton succession in a typical temperate lake. Since its publication, numerous cases showing that this model is not universally applicable and omits some important ecological processes have been put forward (*e.g.*, bacterial loop; O'Sullivan and Reynolds, 2004). Nevertheless, the model provides a coherent conceptual framework for the interpretation of plankton succession, which can be interestingly compared with the observed dynamics. The PEG model refers to warmer lakes where ice-free seasons are longer than in Alpine lakes and fish are naturally present. The turnover of the crustacean zooplankton species usually observed in temperate lakes is time-limited in Alpine lakes, where large crustaceans have enough time just for one generation and are not affected by fish predation. If we could imagine to condense the PEG model in a shorter ice-free season and to remove from the model the effect of fish, it is likely to provide something very similar to the summer succession of NIV and TRS. However, further efforts and intercomparisons are needed to understand how the successional scheme described for NIV and TRS can be applicable to other Alpine lakes and to include the winter, under-ice dynamics.

## CONCLUSIONS

The ecological dynamics of NIV and TRS show very clear initial and final stages, corresponding to ice breakup at the beginning of July and water cooling at the end of summer, respectively. Allogenic environmental fluctuations, together with the short duration of the Alpine summer, are responsible for the fast-paced ecological dynamics observed in the two lakes, which never reach a stationary equilibrium, intended here as a period of little variability of total biomass and community composition (Naselli-Flores *et al.*, 2003).

The rapid succession of events in extreme ecosystems, such as the Alpine lakes studied here, confirm the complexity of the interactions among the different trophic compartments, as well as the important role of external environmental factors. This research underlines the ecological value of seasonal niche partitioning among organisms with different size. In addition, prokaryotes contribute efficiently to the TOC in periods of nutrient limitation or oxygen depletion.

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